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Short Communication

Brain and brain stem harvesting technique in prenatal *Gaddi* sheep fetuses

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Abstract

A modified approach to harvest fetal brain along with brainstem in small ruminants was designed. A total of 54 number of *Gaddi* sheep fetuses were used in the present study. With no marked abnormality, the fetuses were aged between 30 days of gestation until full term. The brain and brainstem was harvested by removal of cranial vault via circular incision around it and cruciform incision along the sutures on its dorsal aspect. The squamous part of occipital bones was removed by wedge shaped incision. The resultant sample can be used for autopsy, neurosurgical, developmental and anatomical studies.

Key words: Brain, removal, brainstem, fetus, sheep, craniotomy.

Fetal neurology is a discipline that will continue to develop rapidly in the near future, making it easier to diagnose any abnormality quickly and reliably (Legido *et al*. 2004).There are many methods for harvesting brain of adult animals. There seems to be very scarce information on removal of brain to study the prenatal development in animals, especially in small ruminants. Keeping this fact in view, the present study was carried out to elucidate the fetal brain and brain stem harvesting methodology from prenatal *Gaddi* sheep fetuses.

The present study was conducted on the brain of 54 *Gaddi* sheep fetuses in the Department of Veterinary Anatomy, DGCN College of Veterinary and Animal sciences, CSKHPKV, Palampur, Himachal Pradesh. The samples were collected from pregnant apparently healthy Gaddi sheep from the slaughter houses in and around Palampur region. The gravid uteri collected were dissected out and the fetuses were exposed by uterine incision. The fetuses were freed from the fetal membranes after removal of cotyledons from caruncles. Immediately after collection, the umbilical cords of these fetuses were ligated and cleaned with cotton soaked in water. The weight of each fetus was recorded with Monopan Electronic Balance and a graduated nylon tape (Harvey 1959) was used for measuring the crown rump length (CRL). The approximate age of fetuses were calculated by putting the CRL values in the formula postulated by (Gall *et al.* 1994) viz.Y = 2.74X + 30.15, where Y is the age of embryos in days and X is the CRL in centimeters. The age of the foetuses collected for present study ranged from early pregnancy to near full term.

The equipments used for this dissection were- forceps (toothed and non toothed), scissors (blunt and sharp), scalpel, chisel, long bladed knife, retractor, bone nibbler and handheld saw.

The earliest evidence of the commencement of skull ossification is 45 days as the basiocciput and exoccipu begin to ossify (Harris 1937). So, the procedure for procurement of brain along with brainstem is divided into two parts, first from 30-60 days of gestation and second part from above 60 days of gestation to full term of the Gaddi sheep fetuses. The method adopted in this study was a coglomeration of the traditional approaches to brain and spinal cord removal in humans (Brash1961) along with removal of squamous part of occipital bone. The skull cap is removed in pieces by cruciform incision on the top and circumferential incision sideways.

Method adopted for fetuses from 30-60 days of gestation

Initially a circular incision was made around the head of the fetus with the help of scalpel. The scalpel was used carefully and gently to avoid excess pressure and brain tissue damage. A midline incision was made

on the semi ossified cranium with the scalp (indistinguishable via naked eye). Thereafter, the frontal part of the semi ossified cranial bone was lifted from front till the occiput with the help of non toothed pointed end forceps. This step is done to each halves of the semi ossified cranium. It exposed both lissencephalic cerebral hemispheres as well as partial mesencephalon of the fetal brain (Fig 1). For the visualization of cerebellar plate and medulla oblongata, the semi ossified dorsal cranial bone was subsequently removed by making two oblique incisions at the occiput (flower vase/wedge shaped) and extended upto the caudal arch of atlas.

Method adopted for fetuses above 60 days of gestation to till term

The fetuses were placed in the supine position on the dissection table. A circular outline was drawn with a skin pencil above the orbit margins, laterally above the auricle, and subsequently 1 cm above the occipital bone. A sharp scalpel along the indicated line freed the scalp enmasse from the cap on the skull. The scalp was removed as a whole or in two parts by mid line incision on it. In case of mid line incision the avulsed scalp was made to hang at the lateral sides in proximity to orbit, temporal lobe and auricle by a pedicle. In the next step, a circular cut by handheld hexa-saw was given through the skull, holding the cutting edge of the saw always on top of the edge of the skin covering the lower part of the head. The calvarium was kept as such. Afterwards, a longitudinal cut was made on the frontal suture and extended upto saggital suture. Perpendicular to this longitudinal cut, another cut was given through the coronal sutures of the skull. The above said cruciform shaped cuts were made with handheld hexa–saw by carefully monitoring the force applied in order to minimize tissue damage of the brain (Fig 2).Then with anon toothed pointed end forceps, the ossified pieces of skull cap were removed one by one to expose the underlying duramater. The duramater attachments were gently cut from the lateral sides and reflected up to the midline. Thereafter, the falxcerbri was cut at its anterior aspect and the frontal lobes of the brain were gently lifted to locate olfactory and optic nerves. The stalk of the pituitary gland was cut after removing the optic nerves which were connecting the orbit. The occulomoter, trochlear and trigeminal cranial nerves were dissected at their entry points into the cavernous sinus. The temporal lobes were teased out and care was taken not to apply excess pulling force on the lobes. The fetus was then turned prune in the next step and the rest of the scalp was cleared, two oblique incisions (wedge shaped) were made from the cutting margin of the squamous part of the occipital bone and stretched to the back arch of the atlas. The spinal cord was cut below the level of first cervical vertebra. Afterwards, the tentorium cerebelli was excised from the wedge by working from the posterior aspect. This exemplified the cerebellum laterally and pons and medulla oblongata ventrally. Thereafter, the remaining cranial nerves were traced and cut by constant traction on the inferior brain surface by using sharp scissors or scalpel. Posterior nuchal muscles were removed and squamous portion of occipital bone was excised with the help of bone nibbler and toothed forceps. The distal aspect of brain was fully revealed along with leptomeninges.

Both longitudinal and transverse craniotomies were most widely used forms of brain extraction in animals. But these have disadvantages such as longitudinal craniotomy taking inordinate amount of time and special instruments. Whilst transverse craniotomy results in transverse slices of brain which due to possible anatomical damage are unsuitable for many studies (Western Australian Agriculture Authority 2015). A study in pigs manifested direct excision of brain without decapitation by hexagonal craniotomy. The above said technique was quicker and far simpler than the longitudinal and transverse craniotomy techniques but has limitation in removal of brainstem and caudal cranial nerves (Bassi *et al*. 2018). The technique suggested in this paper has an exemplary advantage in harvesting the cerebrum, cerebellum and brainstem of prenatal fetuses of varying gestational ages. As the brain of prenatal fetuses was very delicate and friable in nature, the cruciform cut given on the skull cap lead to gentle removal of bones thereby causing least damage to the brain tissue for anatomical and neurological studies. Space besieging lesions in the brain and brain stem were better visualized. The excised brain along with the brainstem can be kept as a laboratory specimen, which can be used for academic and research purposes. This can be useful to students of neurosurgery, orthopaedic and developmental studies.

This methodology provides a great opportunity for young neurosurgeons to train in the management of bleeding and complications from the surgery (Parsak *et al*. 2006). This methodology has plenty of benefits such as the materials used were inexpensive and convenient to obtain. The procedure was concise and economically viable. Needless to say, advanced instruments, technologies and unique facilities were not necessary. Streamlining the fetal brain and brainstem harvesting

method by using the approach designed above is going to encourage the modern research and clinical science including the brain physiology and anatomy.

Conflict of interest: There is no conflict of interest among the authors.

Fig 1. Photograph of Gaddi sheep fetus of 47 days of gestation showing A- Cerebral hemisphere, B- Mesencephalon, C- Occipital part of skull

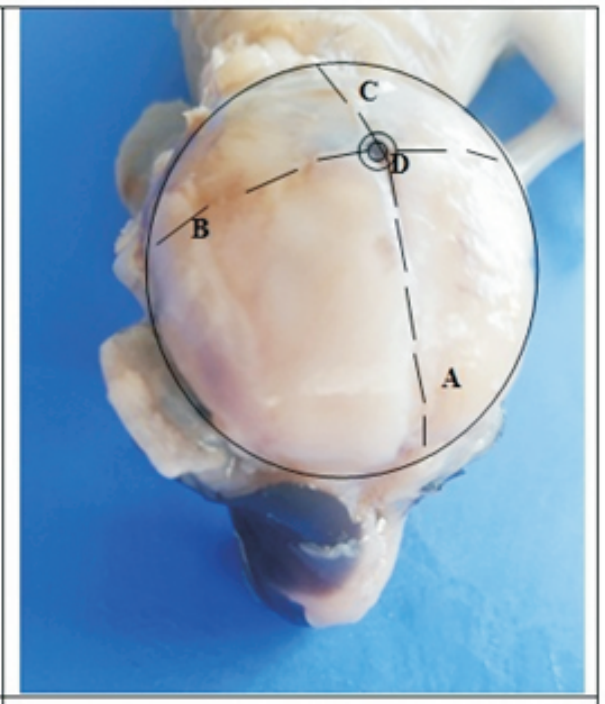

Fig 2. Photograph of skull of Gaddi sheep fetus of 86 days of gestation showing cruciform incision on skull along with circular incision around the calvaria. A- Frontal suture, B- Coronal suture, C- Saggital suture, D- Bregma

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